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Soy isoflavones and phytic acid: Effects on cardiovascular disease risk in postmenopausal women

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Soy isoflavones and phytic acid: Effects on cardiovascular disease risk in postmenopausal women

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GENERAL INTRODUCTION

Thesis Organization

My thesis includes a general introduction about the objectives, hypothesis, specific aims, and significance of our research, followed by a review of literature, a manuscript, and general conclusions. References for chapters 1 and 2 appear at the end of the review of literature, whereas references for chapter 3 appear at the end of the manuscript.

Objectives

The purpose of this study was to determine whether soy protein components, isoflavones and phytate, would reduce the risk of cardiovascular disease (CVD) in postmenopausal women.

Hypothesis

Soy protein components will reduce the risk of CVD in postmenopausal women, specifically that isoflavones will favorably alter blood lipid concentrations and that phytate will decrease oxidative stress indices.

Specific Aims

To determine the effect of phytate and isoflavones from 40 g of soy protein on CVD risk as reflected by circulating lipids and oxidative stress indices.

Significance of Study

Men are usually at higher risk of developing CVD than women; however, after menopause the risk for women becomes similar to that of men. The lack of estrogen affects antioxidant status, as well as lipid and lipoprotein concentrations (Goudev et al. 2000), both of which have been implicated in the pathogenesis of CVD (Saito et al. 2003; Cooke et al.

2003). Evidence indicates that oxidative stress increases and oxidative defenses decrease after menopause, contributing to the rise in CVD.

Soy protein consumption has been shown to significantly decrease serum concentrations of TC, LDL-cholesterol, and triacylglycerols (TG) (Vigna et al. 2000; Scheiber et al. 2001). This cholesterol-lowering activity is generally attributed to the isoflavones in soy. In addition, soy isoflavones may also possess antioxidant properties, protecting against oxidation of LDL (Jenkins et al. 2000a).

Phytate, another component of soy protein, was originally considered an anti-nutrient. However, phytate has been shown to have some health benefits, including lipid-lowering activities (Jariwalla et al. 1990; Klevay 1977). Because of its binding capacity to iron, phytate makes iron unavailable to participate in the Fention reaction for hydroxyl radical formation (Rao et al. 1991), and thus may prevent oxidative stress, such as atherosclerotic lesion formation and lipid peroxidation (Ko and Godin 1991; Porres et al. 1999).

REVIEW OF LITERATURE

Diseases Associated with Aging

Aging is associated with gradual biochemical, physiologic, and functional changes. It may be defined as a cumulative process of damaged body constituents that are not repaired or renewed, leading to malfunction of physiological processes, resulting in death due to internal or external stresses (Goto et al. 1995). Cellular proliferation, declining rates of deoxyribonucleic acid (DNA) repair, free radical damage, lower rates of protein synthesis, and immunologic breakdown are some additional examples of possible alterations that aging individuals experience. The main underlying cause of these physiologic changes is cellular aging, which is the gradual decrease in the ability of a cell to maintain homeostasis. This deterioration in cellular homeostasis is associated with an accumulation of abnormal and oxidatively altered proteins in cells (Vittorini et al. 1999). While there are protective systems to compensate for cellular damage, these defense mechanisms themselves may be compromised. Therefore, aged individuals potentially face many health issues, such as neurodegenerative diseases, CVD, and osteoporosis. However, my thesis focuses mainly on CVD and is discussed below in detail.

Cardiovascular Disease

Cardiovascular disease is one of the leading causes of death among middle-aged men and postmenopausal women. The major underlying cause of CVD is atherosclerosis, a slow, progressive disease in which lesions of plaque build up in the arterial wall as a result of intimal injury. One of the factors that cause injury is an alteration in blood lipids. Stevenson et al. (1993) found that TC, LDL-C, and TG concentrations had positive associations with

age, while a significant negative association was observed between age and high-density lipoprotein cholesterol (HDL-C) concentration.

Risk Factors

There are many contributing factors to CVD, some of which are nonmodifiable. However, most are modifiable with dietary intervention, exercise, medication, or a combination of therapies.

Nonmodifiable

Age is a strong contributor to the CVD risk. The rate of cardiovascular morbid events dramatically increases after the age of 70 (Nevin and Pharr 2002), because activity levels decrease and cholesterol concentrations rise. Also, coronary artery disease (CAD) is very dependent on age in women.

Gender also has an affect on disease risk, whereby men are usually at higher risk of developing CVD than women. After menopause, the risk for women becomes similar to that of men. In postmenopausal women, the lack of estrogen affects antioxidant systems, as well as lipid and lipoprotein concentrations, leading to increased risk of CVD (Goudev et al. 2000). The absence of estrogen also causes adverse changes in vessel walls, as well as unfavorable alterations in prostacyclins, steroids, lipoprotein(a), and growth factors. These factors cause postmenopausal women to experience enhanced cardiovascular stress responses compared to men and premenopausal women (Bairey-Merz et al. 1998). This greater reactivity could augment the development of atherosclerosis, thrombosis, and elicit a myocardial infarction. Additionally, these menopause-related effects appear to be independent of the aging process (Stevenson et al. 1993). Few women recognize their susceptibility to heart disease, and thus delay the identification of disease, making the

prognosis of CVD much less favorable than it is for men. Therefore, many women may have fairly advanced disease by the time a diagnosis is even established (Lewis 2002; Wenger 1997).

A family history of dyslipidemia (elevated TC, LDL-C and TG, low HDL-C) is another strong risk factor for developing CVD. For example, dyslipidemia is highly inheritable and contributes strongly to the development and progression of atherosclerosis. Individuals with genetic disorders, such as familial hypercholesterolemia, in which there is a defect in LDL receptor function, are at high risk for early atherosclerosis and myocardial infarction (Brook et al. 1989). Although heterozygotes, respond well to medication and dietary intervention, individuals who are homozygous do not usually live beyond twenty years of age (Sprecher et al. 1984).

Modifiable

Traditional risk factors for CVD include elevated blood pressure, hypertension, dyslipidemia, smoking, and diabetes mellitus, all of which are amenable to change (Saito et al. 2003). Other modifiable factors, such as poor diet, impairment of glucose tolerance, obesity, and physical inactivity are interrelated and also contribute to the disease. In addition, several new markers of risk have been suggested for use in screening to better identify patients at high risk for cardiovascular events. They include elevated circulating c-reactive protein (CRP), homocysteine, excess iron, and oxidative stress. Circulating concentrations of apolipoprotein A-I, apolipoprotein B-100, lipoprotein(a), and interleukin-6 may also be possible predictors of lesions that lead to the development of CVD and cardiovascular events. Because CVD is still the leading cause of death in men and women, there is an ongoing need to define the most powerful predictors. It is also important to take

into consideration that various cardiovascular factors may not be independent of one another, and may have direct or indirect effects on CVD (Saito et al. 2003). My thesis will mainly focus on the risk associated with unfavorable lipid concentrations, elevated homocysteine and c-reactive protein concentrations, oxidative stress, and iron excess.

Lipid Profile

Lipids play many important physiologic roles. Of most importance is the role of cholesterol in membrane structure and function and the role of lipids in storing energy in adipocytes. Adipose tissue provides a cushion to protect organs and bones from injury. Serum cholesterol and TG concentrations are homeostatically controlled to meet these needs, and the body generally produces the amount required. However, the Western diet provides an abundance of energy and fat, particularly saturated fat, which generally contributes to high cholesterol concentrations.

Alterations in blood lipids, such as increased LDL-C and TG, as well as decreased HDL-C, are all risk factors for CVD. Together, they aid in the formation of atheromatous lesions, which ultimately cause stenosis or occlusion of the arterial lumen (Saito et al. 2003). Numerous studies have indicated that LDL-C concentration should be considered the primary target in the assessment of CVD risk, but HDL-C may also be a critical factor in risk assessment. Because low HDL-C is associated with various other CVD risk factors, particularly cigarette smoking, obesity, and elevated TG, it follows that HDL-C may possibly be a marker when these potential causal variables are present. An eight-year follow-up study on men and women ≥30 years of age revealed that HDL-C was inversely related to CVD and CAD mortality (Jacobs et al. 1990). In a more recent study, Lemieux et al. (2001) performed a metabolic profile (family history, history of smoking, diabetes mellitus, blood pressure,

height and weight, lipid and lipoprotein profile, and electrocardiogram) in 2103 middle-aged men and observed significant differences in average TC/HDL-C ratios among the TG tertiles, as well as elevated TC/HDL-C ratios among the overweight, hyperinsulinemic, and hypertriglyceridemic individuals. For these reasons, cholesterol and lipoprotein concentrations are essential in assessing the risk for CVD.

Homocysteine

Homocysteine is a naturally occurring, sulfur-containing amino acid produces from methionine. Methionine can also be regenerated from homocysteine in a reaction that is dependent on vitamin B_{12} and folate. The normal plasma concentration in healthy individuals is approximately 10 μmol/L (Hoffer et al. 2001). However, this concentration is greatly influenced by age, gender, and medication, as well as genetic, nutritional, and pathologic factors. Plasma homocysteine increases with age and is higher in men than in women (Warren 2002). Additionally, premenopausal women have lower plasma homocysteine than postmenopausal women, and estrogen therapy (ET) appears to decrease concentrations (De Leo et al. 2000), suggesting that plasma homocysteine may also be related to estrogen status. Other major lifestyle determinants of plasma homocysteine include coffee consumption, smoking (Nygard et al. 1998), and end-stage renal disease (Hoffer et al. 2001).

Homocysteine concentrations are inversely related to concentrations of vitamins B_6, B_{12}, and folate. A deficiency in one or a combination of these nutrients can lead to hyperhomocysteinemia. This elevated plasma homocysteine concentration may significantly increase the risk of CAD and stroke and is thought to be an independent risk factor for CVD (Bautista et al. 2002). Stampfer et al. (1992) assessed this association in men and found that

moderately high plasma homocysteine was, in fact, associated with subsequent risk of myocardial infarction, independent of other coronary risk factors. Similarly, Ridker et al. (1999) reported that elevated homocysteine, independent of several other cardiovascular risk factors, moderately increased three-year risk of CVD in postmenopausal women. Similarly, in men and women ≤60 years of age, an increased plasma total homocysteine concentration conferred an independent risk of vascular disease, similar to that of smoking or hyperlipidemia (Graham et al. 1997). In addition to being a secondary consequence of B_6, B_{12}, and folate deficiency, very high plasma homocysteine also results from a rare genetic disorder of impaired homocysteine metabolism resulting in homocysteinuria. A characteristic feature of this condition is high circulating homocysteine concentrations, as well as severe, premature arteriosclerosis (Stampfer et al. 1992) and osteoporosis (Lubec et al. 1996).

There are many possibilities to explain the relationship between homocysteine and CVD. A widely researched mechanism is the involvement of homocysteine in oxidative damage. Homocysteine exerts toxicity on endothelial cells by increasing hydrogen peroxide (H_2O_2) production, causing stress on antioxidant defense systems, and promoting lipid peroxidation (Huang et al. 2001). As a consequence, blood vessels lose their elasticity and ability to dilate, resulting in greater intimal injury (Warren 2002). Calcium, cholesterol, and collagen then adhere to the sites of intimal injury and form atherosclerotic plaques (Warren 2002). Thus, by damaging the endothelial lining of vessels, homocysteine contributes to atherosclerotic plaque development. Tofler et al. (2002) observed another possible mechanism, in that elevated homocysteine concentrations were linked to a decrease in fibrinolytic potential, thereby suggesting a prothrombotic effect. By reducing the production

of nitric oxide (NO) and prostacyclin, homocysteine interferes with the platelet-endothelium interaction. Also, by enhancing endothelial factor V activity and reducing the activity of thrombomodulin, homocysteine promotes clotting (De Leo et al. 2000). Elevated homocysteine has also been shown to stimulate the proliferation of vascular smooth muscle cells and impair endothelial-dependent vasodilatation (Tofler et al. 2002), thus suggesting another mechanism for the effect of homocysteine on CVD.

C-Reactive Protein

Evidence from recent studies has shown that inflammation plays a strong role in the pathogenesis of atherosclerosis. Inflammatory components are believed to contribute greatly to instability and rupture of atheromatous plaque, thus causing atherothrombotic events (Saito et al. 2003). A variety of biomarkers have been shown to predict risk of future cardiovascular events, including interleukin-6 and cellular adhesion molecule (Ridker 2003). However, most attention has focused on CRP, an acute-phase protein and commonly assayed marker of inflammatory and infectious responses. The liver produces CRP in response to cytokines, particularly interleukin-6. C-reactive protein induces expression of cellular adhesion molecules and mediates LDL uptake by macrophages (Ridker 2003). Circulating concentrations of CRP are stable within individuals over long periods of time, thus reflecting the chronic nature of the inflammatory process (Saito et al. 2003). Also, compared to other circulating markers of inflammation, CRP seems to have the most consistent relation to CVD risk in a variety of clinical settings (Saito et al. 2003).

A recent study examined healthy postmenopausal women over a period of three years to assess the risk of cardiovascular events associated with baseline concentrations of CRP and other markers of inflammation. The concentration of CRP was the most significant

predictor of risk for cardiovascular events, even in women with LDL-C concentrations below 130 mg/dL (Ridker et al. 2000). Another study found that minimally elevated serum CRP concentrations for at least six years were associated with increased mortality from CVD among older women (Tice et al. 2003). Based on these results, CRP has been proposed as an independent risk factor for CVD, myocardial infarction, stroke, and vascular death, especially among those who have hyperlipidemia and/or a heightened inflammatory response. Unfortunately, the mechanism and pathophysiology explaining the relationship between CRP and CVD is not known. It may be that CRP indirectly contributes to foam cell formation by promoting leukocyte adhesion to endothelial cells, resulting in enhanced recruitment of monocytes to atherosclerotic plaques (Niessen and Hack 2003; Tice et al. 2003).

Aside from the current interest in the role of CRP as an independent predictor of CVD, there is controversy about its direct function. While many studies suggest that CRP is an independent risk factor for CVD, the association may partly reflect mutual associations with already established CVD risk factors. C-reactive protein is associated with various risk predictors of CVD, including fibrinogen, plasma viscosity, and white cell count, all of which (like CRP), are associated with reactions mediated by cytokines, such as interleukin-6 (Woodward et al. 2003). C-reactive protein was also significantly positively associated with body mass index, serum TC, and TG, but negatively associated with serum HDL-C (Woodward et al. 2003). Hence, it may be that the role of CRP in the pathogenesis of CVD is through its association with the above hematological factors. Yet, no matter how it is associated with CVD, inflammatory markers, such as CRP, may improve CVD prediction when used in conjunction with traditional risk factors (Saito et al. 2003).

As a result, researchers have evaluated strategies for expanding the routine screening approach to include CRP (Ridker 2003). However, there are limitations in that elevated CRP concentrations may not always be predictive of CVD risk. For example, CRP concentrations increase rapidly in response to injury, infection, inflammatory stimuli, and other disease conditions. It is also a marker of biologic aging (Kushner 2001) and menopause (Woodward et al. 2003). In addition, Saito et al. (2003) found that subjects treated for hypertension, diabetes mellitus, and CAD had increased CRP. Overweight adults may also have elevated CRP, largely due to adipose tissue and its ability to secrete interleukin-6 (Saito et al. 2003). Other states in which minor CRP elevations have been reported include chronic fatigue, high protein diets, depressive symptoms, and mild viral infections (Kushner and Sehgal 2002). Additionally, CRP concentrations fluctuate substantially from day to day, which may lead to considerable misinterpretation in this inflammatory marker with respect to its association with CVD.

Oxidative Stress

In healthy individuals, there is a balance between pro-oxidants and antioxidant defenses. Antioxidants significantly delay or inhibit the oxidation of substrates, thus detoxifying harmful oxidants. The major intracellular antioxidant enzymes involved in removing free radicals are superoxide dismutase (SOD), glutathione peroxidase, catalase, and glutathione reductase. Extracellular factors include vitamins E and C. Additional extracellular antioxidants include albumin, which inhibits copper-stimulated peroxidation of erythrocyte membranes, and uric acid, which may scavenge singlet oxygen, peroxyl radicals, and hydroxyl radicals (•OH) (Chanarat 1992). Table 1 lists the major antioxidants that protect against free radical reactions.

Table 1. Antioxidant protection against free radical reactions in vivo[1]

Intracellular Factors	Extracellular Factors
Superoxide dismutase (SOD)	Ceruloplasmin
Catalase	Albumin
Glutathione peroxidase	Lactoferrin
Methionine sulphoxide reductase	Extracellular SOD
	Urate
	Vitamin C
	Vitamin E
	Hyaluronic acid

[1] Chanaratet 1992

Vitamin E is a poor antioxidant in the cytoplasm, but its antioxidant property is extremely useful when associated in the membrane (Gutteridge 1995). Because of its lipophilic characteristics, it protects membranes against lipid peroxidation by scavenging lipid peroxide. Vitamin C acts by scavenging H_2O_2, as well as the superoxide ($O_2^{-}\bullet$) and •OH (Chanarat 1992). Other antioxidant enzyme systems are involved in promoting the synthesis of various antioxidants and in preventing the actual formation of reactive oxygen species (ROS).

Antioxidants combined may have an additive effect in protecting against oxidative stress. Goudev et al. (2000) found that a combined-antioxidant supplementation in postmenopausal women for 12 weeks inhibited free radical formation, and decreased serum concentrations of cellular adhesion molecules, which are markers of arterial wall

inflammation. Ultimately, antioxidants protect against excessive free radical generation and the consequences thereof (Rice-Evans 1995). They are present throughout the body, including in the interstitial fluid of the arterial wall, where lipoprotein oxidation is thought to occur (Kontush and Beisiegel 1999). Hence, depletion of antioxidant capacity increases oxidative stress and damage.

Table 2. Sources of reactive oxygen species-free radicals in humans[1]

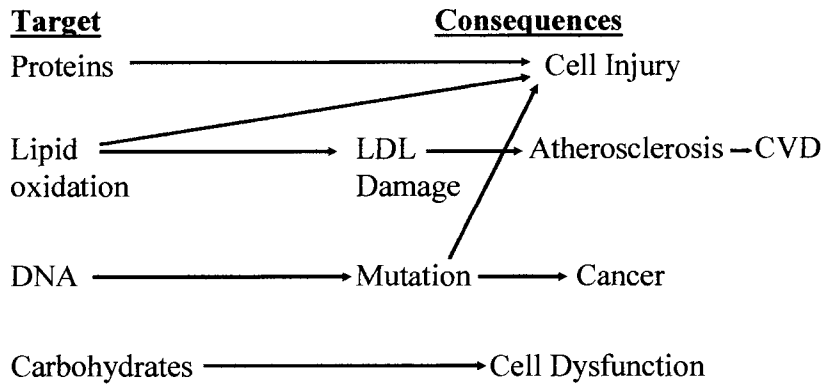
Physical	Biological
Ionizing radiation	Inflammation
Ultraviolet	Ischemia/reperfusion
	Endogenous sources
Chemical	
Carcinogens/drugs	Transition metals
Tobacco smoke	Iron
	Copper

[1] Okada 1996

A disruption in the balance between pro-oxidant and antioxidant systems in cells may be defined as oxidative stress, which has also been implicated in the pathogenesis of cancer, CVD, and many neurodegenerative conditions, including Alzheimer's disease, Huntington's disease, and Parkinson's disease (Cooke et al. 2003). Individuals are exposed to an array of exogenous sources of oxidative stress, including irradiation, drugs, and toxic, mutagenic, and carcinogenic chemicals. Oxidative stress may also originate from the diet or from normal physiological processes or pathological states. Table 2 lists various sources of oxidants.

Polyunsaturated fatty acids, proteins, DNA, and carbohydrates are all susceptible to attack by oxidants (Chanarat 1992), and this damage accumulates with age (Packer 1995). Over time, endogenously generated free radicals may cause cellular damage leading to gradual deterioration, dysfunction, and disease states associated with aging (Cooney and Mordan 1995). Figure 1 illustrates the potential consequences of such oxidative damage.

Figure 1. Free Radical Damage



Free radicals are atoms or molecules that have one or more unpaired electron(s). The radicals originate from exogenous or endogenous sources and are generally unstable and very reactive. While they are essential to many beneficial physiological processes, such as signal transduction and gene transcription (Fang et al. 2002), they also act as highly deleterious, cytotoxic oxidants if produced in excess of normal requirements (Rice-Evans 1995). The imbalance of electrons makes them highly reactive with other compounds, as well as with each other. Further, when defense mechanisms have been compromised, free radicals inflict damage throughout the cell and the surrounding tissue (Kilgore and Lucchesi 1995). Many chronic diseases are initiated by this oxidation of cellular components. Additionally, they may be related to the process of aging.

Table 3 displays the various ROS, including radical and non-radical forms. Reactive oxygen species formulate from exposure to chemicals, drugs, radiation, high oxygen, and normal physiological processes. The $O_2^{-}\bullet$ anion is produced when oxygen molecules react with catecholamines, as well as when electrons leak from the electron transport chain

Table 3. Reactive Oxygen and Nitrogen Species[1]

Reactive Oxygen Species		Reactive Nitrogen Species	
Radicals	Non-radicals	Radicals	Non-radicals
Superoxide	Ozone	Nitric oxide	Nitrous acid
Hydroxyl	Singlet oxygen	Nitrogen dioxide	Peroxynitrite
Hydroperoxyl	Hypochlorous acid		Alkyl peroxynitrite
Alkoxyl	Hydrogen peroxide		
Peroxyl			

[1] From Groff and Gropper 2000

in the mitochondria. It is a precursor for a number of ROS, including H_2O_2, the hydroperoxyl radical, and the hydrogen dioxyl radical. Superoxide dismutase eliminates $O_2^{-}\bullet$ radicals from cells before they elicit damage, but unfortunately, H_2O_2 is generated in the process. Hydrogen peroxide can be converted to water by glutathione peroxidase or catalase, but the rate of H_2O_2 production by SOD is much faster (Gutteridge 1995). Thus, H_2O_2 is available to react with still existing $O_2^{-}\bullet$ radicals to produce •OH, the most potent and reactive of the ROS. Damage by •OH is very site-specific and often produces products that cannot be regenerated by normal cellular metabolism. Unfortunately, this radical lacks a specific enzyme-based defense system to scavenge its existence (Morris et al. 1995). The

singlet oxygen is an additional radical formed by the oxidation of other ROS, as well as from lipid peroxidation of membranes, enzymatic reactions, and photochemical reactions.

Free radicals that contain nitrogen are identified as reactive nitrogen species (Table 3). They are comprised of NO and nitrogen dioxide, which, similar to ROS, can also be converted to non-radicals, such as peroxynitrite. Nitric oxide is an enzymatically-generated free radical and is synthesized in a variety of cells. It is one of the most widespread signaling molecules and participates in virtually every cellular and organ function (Fang et al. 2002). It is extremely important in metabolic processes, including smooth muscle cell relaxation, proliferation of platelet aggregation, neurotransmission, and immune regulation (Fang et al. 2002). On the other hand, NO can also be harmful. During inflammation, macrophages and neutrophils release NO, which acts as a precursor for oxidants such as peroxynitrite, nitrogen dioxide, and the •OH, causing deleterious effects.

Polyunsaturated fatty acids are highly susceptible to free radical attack (Rice-Evans 1995). Their ensuing oxidation primarily results in lipid peroxidation, a radical-initiated chain reaction that is self-propagating in cellular membranes. An initiating free radical extracts a hydrogen atom from one of the polyunsaturated fatty acids, producing a lipid radical (Fang et al. 2002). Molecular oxygen then reacts with the lipid radical, yielding a lipid peroxy radical, which further propagates the peroxidation chain reaction by removing a hydrogen atom from an adjacent lipid to form lipid hydroperoxide (Fang et al. 2002). Chain propagation continues until scavenged by antioxidants or when terminated by the combination of two lipid peroxyl radicals to form less harmful products (Esterbauer 1995). The following reactions illustrate the steps involved in lipid peroxidation.

Figure 2. (Esterbauer 1995)

$LH + X^{\bullet} \rightarrow L^{\bullet} + XH$ (Initiation)

$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$ (Lipid Peroxy Radical Formation)

$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$ (Chain Propagation)

$LOO^{\bullet} + AOH \rightarrow LOOH + AO^{\bullet}$ (Antioxidant Scavenging)

$LOO^{\bullet} + LOO^{\bullet} \rightarrow$ non-radical product + O_2 (Termination)

Lipid peroxidation is often an isolated event within cellular membranes, causing profound effects on membrane function. The lipid peroxy radicals formed during the modification of the polyunsaturated fatty acid side-chains of lipids amplify lipid peroxidation, oxidize cholesterol, react with proteins, and impair functions of enzyme and receptor systems (Rice-Evans 1995). The consequences that result from the peroxidation of membrane lipids include loss of polyunsaturated fatty acids, decrease in lipid fluidity, alteration of membrane permeability, adverse effects on membrane-associated enzymes and receptors, altered ion transport, release of material from subcellular compartments, and generation of cytotoxic metabolites of lipid hydroperoxides (Rice-Evans 1995).

In addition to its deleterious effect within cellular membranes, lipid peroxidation products are carried by plasma lipoproteins, thus leading to the oxidation of lipoproteins, especially LDL (Kontush and Beisiegel 1999). Oxidation of LDL is a vital step in the pathogenesis of atherosclerosis, contributing to arterial wall inflammation and promoting leukocyte adhesion to endothelial cells (Goudev et al. 2000). Oxidized LDL are taken up by macrophages and enlarged to form lipid-laden foam cells that become trapped in blood vessel walls. The result is a massive accumulation of foam cells, known as atherosclerotic plaques or lesions, in the subendothelial space of arteries (Esterbauer 1995). Additionally, oxidized LDL contain cytotoxic lipid peroxidation products that may cause constant irritation to the

endothelial cell layer and provoke other detrimental effects, such as endothelial cell death, platelet aggregation, release of growth factors, accumulation of inflammatory cells, and increased infiltration of LDL (Rice-Evans 1995). Progression of these events may eventually lead to cardiovascular events, such as myocardial infarction or stroke.

Proteins are prone to damage by oxidative stress by various mechanisms. Reactive oxygen species that attack amino acids on proteins may break peptide bonds in the backbone or disrupt the structure of the protein, eliciting changes in secondary or tertiary structures, leading to premature degradation. Denaturation of protein can impair enzymatic activity and modify membrane and cellular functions, including intracellular transport and metabolism (Rice-Evans 1995). Another protein modification is the conversion of side chains of amino acid residues, such as arginine and lysine, resulting in the formation of protein-bound carbonyl groups. These develop more rapidly than lipid peroxidation products and take much longer to degrade.

Pro-oxidants interfere with normal metabolism by oxidizing DNA, resulting in mutation. Reactive oxygen species, such as •OH, extract hydrogen from the purinic and pyrimidinic bases of nucleic acids (Boveris et al. 1999) and cause single and double strand damage (Bohr et al. 1995). The •OH also reacts with DNA by adding double bonds of DNA bases (Cooke et al. 2003). Extensive oxidative DNA damage may result in cytotoxicity, mutagenicity, and gene rearrangements, which may alter cellular phenotype (Bohr et al. 1995) and be responsible for genetic defects. Additionally, DNA mutation is a crucial step in carcinogenesis and is likely to play an important role in the pathogenesis of other diseases as well (Cooke et al. 2003).

Iron Excess

Iron is an essential nutritional element in life. It is a component of hemoglobin and myoglobin, as well as a component of enzymes that are necessary for normal cell function. Iron also catalyzes critical redox reactions needed for many physiological functions. For example, it is involved in energy production, and as part of ribonucleotide reductase, it is involved in DNA synthesis (Orino et al. 2001). Two thirds of the body's iron is found in the oxygen-carrying protein, hemoglobin, with smaller amounts in myoglobin and various enzymes (Gutteridge 1995). The bound iron is transported in the body by transferrin, while excess iron is stored as ferritin and hemosiderin (Emerit et al. 2001; Gutteridge 1995). Ferritin has the capacity to sequester a vast quantity of iron and the ability to maintain it in a nontoxic form (Emerit et al. 2001) and available for use, if needed. In healthy individuals, the excess iron pool is small but can be slightly elevated in people at risk for iron overload, such as in hemochromatosis and conditions of tissue damage. Excess iron accumulates in organs, such as the heart and blood vessels, and may catalyze pathologic reactions involving free radical generation, which in turn leads to cell death and organ dysfunction (Horwitz 1999). Iron has also been found to be elevated in a number of degenerative diseases of aging, such as Parkinson's disease, Alzheimer's disease, and CVD (Walter et al. 1999).

It has recently been suggested that excess iron indirectly increases the risk of CVD by increasing oxidative stress (Salonen 1993; Horwitz and Rosenthal 1999). In vitro, free iron catalyzes the Haber-Weiss reaction, wherein the $O_2^{-}\bullet$ anion and H_2O_2 react to generate a •OH (Figure 3) (Emerit et al. 2001; Morris et al. 1995). Iron also participates in the Fenton reaction, reducing H_2O_2 to a •OH (Walter et al. 1999). Such reactions cause oxidative damage and may enhance the risk for disease. Iron additionally catalyzes the chain

Figure 3. Haber-Weiss and Fenton Reactions

Haber-Weiss reaction (Morris et al 1995)

$O_2^{-}\bullet + H_2O_2 \rightarrow O_2 + HO^{-} + \bullet OH$

Fenton reaction (Emerit et al. 2001)

$Fe^{3+} + O_2\bullet^{-} \rightarrow Fe^{2+} + O_2$

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{-} + \bullet OH$

reaction of lipid peroxidation (van Jaarsveld and Pool 2002), thus producing oxidized LDL (Naimark et al. 1996). Iron also elicits smooth muscle cell proliferation, which indirectly increases the susceptibility of LDL to oxidation by reducing antioxidants in plasma, thus promoting atherogenesis by affecting blood lipid concentartions (van Jaarsveld and Pool 2002). Kiechl et al. (1997) addressed the connection between serum ferritin concentrations and the progression of carotid atherosclerosis in a random sample of 826 men and women 40 to 79 years of age. They found strong evidence that iron promotes lipid peroxidation and thus may cause atherogenesis. High iron stores also down-regulate NO synthesis by the arterial wall, which in turn is associated with atherosclerosis. In a study of females aged 14 to 19, NO concentrations increased gradually as iron deficiency progressed, but then significantly decreased after hemoglobin concentrations were normalized by iron supplementation (Choi et al. 2002). Iron-mediated processes are also capable of causing myocardial injury during reperfusion. Hydrogen peroxide and the $O_2^{-}\bullet$ radical are generated during ischemia and reperfusion and are usually weak oxidants. But, in the presence of iron, these radicals can cause production of •OH (Horwitz and Rosenthal 1999) and cause damage.

On the contrary, other studies have found no association between ferritin and CVD, thus other factors most certainly contribute to the increased risk of myocardial infarction.

Williams et al. (2002) progressively evaluated CVD measures in individuals and found an association between serum ferritin and both anthropometric and metabolic biochemical risk factors. Serum ferritin was also strongly associated with CRP in women, thus suggesting that ferritin may be a surrogate measure of inflammation in women. However, this association could be due to infection, because ferritin concentrations rise in response to acute infection to protect against proliferation of microorganisms (Williams et al. 2002). Therefore, it may be that infection and inflammation contributes to the pathogenesis of atherosclerosis.

Preventive Approaches

The human body provides many protective mechanisms against cellular injury and disease. Hormones and antioxidants both play a part of the body's defense system. However, diet and physical activity also play a role in reducing the risk of disease.

Hormone Therapy

Many women choose to take hormone therapy (HT) as a way of relieving menopausal symptoms. Hormone therapy may also have several potentially beneficial vascular effects, including reductions in LDL-C, increases in HDL-C, arterial dilation, and reductions in platelet aggregation (Nevin and Pharr 2002), as well as favorable increases in insulin sensitivity and antioxidant activities. Hence, if the protection of estrogen is lost when women become estrogen-deficient after menopause, it follows that ET or estrogen plus progesterone (EPT) would minimize susceptibility to CVD and other associated diseases (Sullivan 2003).

Unopposed Estrogen Therapy

In the mid 1990's, evidence indicated a major role for ET in the primary and secondary prevention of CVD (Nevin and Pharr 2002). A nine-week administration of oral

estrogen in postmenopausal women showed that ET also improved flow-mediated endothelium-dependent vasodilatation (Lieberman et al. 1994). The postmenopausal estrogen/progestin intervention trial (PEPI 1995) investigated the effects of different hormone regimens and revealed that estrogen alone or estrogen with progestin improved lipoproteins and lowered fibrinogen concentrations; however, the unopposed estrogen had more favorable effects on HDL-C status. The mechanism by which ET elicits cardioprotective effects is still unclear. Proposed mechanisms include changes in lipid metabolism, blood pressure, carbohydrate metabolism, coagulation factors, and endothelial function (PEPI 1995).

Despite the beneficial findings above, recent randomized clinical trials have shown that estrogen therapy may not always be optimal for treating or preventing medical conditions associated with the aging process (Nevin and Pharr 2002). Disadvantages of ET include potential increases in the risk of breast cancer, vaginal bleeding, and endometrial cancer (Glazier and Bowman 2001). Estrogen has also been shown to raise TG concentrations as a result of an increase in VLDL production (LaRosa 1998). These risks, as well as the benefits, vary from individual to individual; therefore, it is not always clear that ET is the best option to combat menopausal symptoms and consequences.

Estrogen plus Progesterone

The EPT regimen is an additional pharmacological therapy option that effectively reduces the symptoms of menopause, although the primary role of progesterone is to protect the endometrium from hyperplasia and cancer. EPT has also been shown to reduce the concentration of cellular adhesion molecules (Goudev et al. 2000), as well as to produce favorable effects on circulating lipoproteins, particularly by lowering LDL-C concentrations

(LaRosa 1998). In addition, it may reduce the activity of osteoclasts and thus slow bone loss.

In contrast, many studies have shown that EPT does not reduce the prevalence of atherosclerosis nor any other events associated with CVD (Sullivan 2003). In the Heart and Estrogen/Progestin Replacement Study (HERS), treatment with oral EPT did not reduce the rate of CAD events in postmenopausal women with established coronary disease. In addition, the EPT increased the rate of thromboembolic events and gallbladder disease (Hulley et al. 1998). Also, the medical community is very concerned about the effect of EPT on breast cancer risk. Schairer et al. (2000) found that an EPT increased breast cancer risk beyond that associated with estrogen alone during a fifteen-year follow-up study in postmenopausal women. Also, recently, the EPT arm of the Women's Health Initiative was halted due to evidence of increased risk of invasive breast cancer, as well as increased risk of CAD, stroke, and pulmonary embolism in women (WHI 2002). Many prospective studies also suggest an early increase in cardiovascular events following the initiation of EPT. The HERS study reported that EPT appeared to increase the risk for primary CAD events in the first year of therapy, but this decreased in subsequent years (Hulley et al. 1998). In addition, EPT may result in a three-fold increase in venous thromboembolism, as well as risks for ovarian cancer and asthma (Nevin and Pharr 2002). Although minimal in comparison to the limitations noted above, EPT is also associated with adverse effects, such as uterine bleeding. Thus, the balance between the risks and benefits of EPT appear to be ever evolving and perhaps somewhat contradictory depending upon the study. Nonetheless, HT is not indicated or approved for reducing CVD risk in postmenopausal women.

Soy Protein

Many adults take a preventive approach by identifying risk factors and establishing lifestyle patterns that promote positive health and well being. Diet and physical activity play a major role in reducing the susceptibility to disease. Populations in Asian countries consume significant daily amounts of soy protein in products such as soymilk, tofu, and tempeh (Erdman 1995). Coincidently, the incidence of CVD is lower in these countries than in Western nations (Glazier and Bowman 2001). Despite this fact, some Americans are still cautious of consuming soy protein. They believe that soy poses potential risks for enhanced breast tumor growth and abnormal sexual development, as well as adverse effects on the central nervous system. To assess hormone-related risks, Jenkins et al. (2000a) examined the consumption of soy protein foods in thirty-one hyperlipidemic men and postmenopausal women. They found no evidence of increased urinary estrogenic activity, a marker for hormone-dependent cancers, suggesting little or no increase in the risk for adverse health effects. Also, while allergic reactions to soy products may exist, they vary between individuals and are very rare in adults. Many Asian populations typically consume 20 g of soy protein daily (Erdman 1995) and have done so for hundreds of years, implying that soy protein consumption is safe. In addition, soy protein has been successfully used as a protein source in infant formulas. Safety, however, is not the only issue causing the low acceptance of soy protein in Western countries. The beany or bitter aftertaste of many soy foods prevents acceptance by many individuals and does not bring about the same positive response as "meat," "eggs," or "milk" (Erdman 1995). In order to encourage and increase the addition of soy foods into the Western diet, the public will need to be educated about the benefits of soy protein.

The potential benefits associated with soy consumption include a decreased risk of several cancers, including breast, prostate, and colon, as well as CVD and osteoporosis. Scheiber et al. (2001) investigated the effect of soy protein on markers of both CVD and osteoporosis in normal postmenopausal women and revealed that the consumption of soy foods, containing approximately 60 mg of isoflavones, for 12 weeks resulted in significant reductions in risk factors of both CVD and osteoporosis. Subjects had significantly higher serum HDL-C concentrations, as well as increased lag-time for LDL peroxidation after intervention. Plasma total homocysteine concentrations have also decreased with the addition of 30-50 g of soy protein per day to a lipid-lowering diet (Tonstad et al. 2002). In addition, a meta-analysis by Anderson et al. (1995) concluded that soy protein, in substitution for animal protein, produces significant decreases in serum concentrations of TC, LDL-C, and TG. However, this decrease in cholesterol via consumption of soy protein varied according to initial cholesterol concentrations. This greater lipid-lowering effect of soy seen in hypercholesterolemic subjects, compared to that in normolipidemic subjects, has also been documented in other studies (Vigna et al. 2000; Tonstad et al. 2002).

Other possible benefits of soy protein with isoflavones include relief of menopausal symptoms. Japanese women generally have delayed menopause and reduced symptoms, perhaps due to their high consumption of soy products (Sirtori 2001). However, some studies have found little or no effect in the number of hot flushes in postmenopausal women consuming a soy protein enriched diet (Vigna et al. 2000; St. Germain et al. 2001). Thus, the interindividual variability in response makes results unpredictable.

Many mechanisms have been proposed as being responsible for the hypocholesterolemic effect of soy protein. A popular hypothesis is that it mimics the effect

of statin drugs (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors [HMG-CoA reductase]) and up-regulates LDL receptors. Low-density lipoprotein receptors are chronically depressed in hypercholesterolemia, and soy protein may exert an up-regulation of LDL receptors, thus lowering LDL-C. The 7S globulin, one of the major storage proteins of soybeans, may be responsible for this direct up-regulation of LDL receptors. Lovati et al. (1992) found that this soybean globulin could effectively induce LDL receptors in a human liver cell line derived from normolipidemic subjects. However, this up-regulation may also be due to alterations in hormone concentrations. Soy consumption in animals has been shown to increase thyroxin and thyroid stimulating hormone (Forsythe 1990), thus increasing LDL receptor activity, HMG-CoA reductase activity, and bile acid excretion (Potter 1995). Additionally, a high insulin-to-glucagon ratio that is associated with an increased risk of CVD decreases after feeding soy protein (Ham et al. 1993). Another possible mechanism may be that soy protein alters bile acid excretion by increasing fecal excretion of bile acids, thus causing the removal of cholesterol from the bloodstream to be used for enhanced bile acid synthesis (Potter 1995). Although this mechanism would result in a reduced concentration of blood cholesterol, it has only been consistently documented in animals (Huff and Carrol 1980).

Interest has also centered on finding the component of soy that is most responsible for its cholesterol-lowering ability. Soy components identified as being likely to have protective effects include saponins, sphingolipids, phenolic acids, phytate, and trypsin inhibitors. Also, soy has a low lysine-to-arginine ratio. Arginine is less hypercholesterolemic than lysine and thus may be responsible for the reduction in serum cholesterol. Although all of these components have been researched, most attention has focused on soy isoflavones. Studies

have also shown that phytate has some beneficial effect on disease prevention (Shamsuddin 1995; Jariwalla et al. 1990); however, it has received less attention compared to the other soy components.

Soy Isoflavones

Phytoestrogens are plant estrogens that occur naturally in plants, fruits, and vegetables. They are very similar to selective estrogen receptor modulators and thus have the potential to deliver the beneficial effects of HT without the undesired health risks (Scheiber et al. 2001). The three main types of phytoestrogens are coumestans, lignans, and isoflavones, the latter of which are the most potent and most investigated (Glazier and Bawman 2001) and are found primarily in soybeans. There are three major varieties of isoflavones (genistein, daidzein, and glycitein), but genistein and daidzein are the two most commonly studied. Isoflavones have been researched mainly because of their relationship with CVD, cancer, osteoporosis, and menopausal symptoms. Reported actions include cholesterol-lowering, thyroxin, and antioxidant activities, as well as enhanced arterial compliance (Nicolosi et al. 2001). Isoflavones also possess weak estrogenic activity (Jenkins et al. 2002) and are structurally similar to human estrogen in that they possess a phenolic ring that enables them bind to estrogen-α and estrogen-β receptors in humans (Glazier and Bowman 2001). However, isoflavones compete with estradiol for these binding sites, thus acting as estrogen antagonists in some tissues.

The effect of soy protein on reducing hypercholesterolemia is mostly attributed to the presence of isoflavones. Goodman-Gruen and Kritz-Silverstein (2001) investigated usual isoflavone intake from diet in postmenopausal women and found that it was inversely related to obesity, fasting, and post challenge insulin, as well as positively associated with HDL-C

concentrations. The Framingham Offspring Study also associated phytoestrogen (isoflavones and lignans) intake with a favorable cardiovascular risk profile (de Kleijn et al. 2002). However, some studies have found no effect of isoflavones on blood lipids. A 55-mg isoflavone supplement given to men and postmenopausal women for 8 weeks elicited no improvement in serum lipid concentrations (Hodgson et al. 1998). Additionally, Hsu et al. (2001) found that supplementing 150 mg of isoflavones per day for 6 months to normal postmenopausal women educed no significant change in plasma lipids. Therefore, the effect of isoflavones on serum lipids in humans remains unclear, and these discrepancies may be attributed in part to interindividual variability or initial cholesterol concentrations. Also, adding 10 mg and 73 mg isoflavone-rich soy protein to a low-fat diet for 3 months in hyperlipidemic men and postmenopausal women both reduced blood lipids, homocysteine concentrations, and blood pressure (Jenkins et al. 2002), suggesting that isoflavones may not be the protective component in soy.

In addition to altering blood lipids, isoflavones may also affect CVD by improving arterial compliance, vascular function (Sirtori 2001), and antioxidant status, the later of which may be due the ability of isoflavones to quench free radicals in vitro, thus donating hydrogen atoms to free radicals and making them less reactive (Mitchell et al. 1998). Many studies show that genistein and daidzein protect against oxidation of LDL. Jenkins et al. (2000b) found that the consumption of soy breakfast cereals in 25 hyperlipidemic men and postmenopausal women reduced oxidized LDL. Also, following a 12-week intervention with soy foods high in isoflavones, Scheiber et al. (2001) found that serum daidzein, genistein, and total isoflavone concentrations were positively correlated with LDL oxidation lag time in postmenopausal women. In addition, the consumption of readily available soy foods has

been also associated with reduced circulating oxidized LDL (Jenkins et al. 2000a). Based on these effects on LDL oxidation, one may postulate that consuming soy protein, specifically isoflavones, may reduce the progression of atherosclerosis.

Phytic Acid

Phytate (inositol hexaphosphate, IP_6) is a naturally occurring compound found in legumes, cereals, whole grains, seeds, and nuts. As a constituent of fibrous products, phytate is thought to contribute to the prevention of cancer and other chronic diseases. It is also found in human cells as IP_6 and its metabolites, where it is important for many cellular processes. Less phosphorylated forms of phytate possess similar characteristics as IP_6, such as its ion-chelating ability (Miyamoto et al. 2000), and are responsible for many of phytate's cellular functions. Unfortunately, the absorption, metabolism, tissue distribution, and excretion of phytate in humans is not clear. Recently, Grases et al. (2001) found that the level of plasma and urinary IP_6 depended on dietary phytate intake, suggesting that a small amount of absorption may occur in humans. Conversly, in rats, when administered with drinking water, IP_6 is rapidly absorbed through the stomach and upper small intestine (Sakamoto et al. 1993).

A considerable amount of attention is being paid to the anticancer and anti-CVD activities of phytate. There are many proposed actions, but most important is phytate's ability to bind to metals. Its unique structure of phosphate groups gives it tremendous potential to chelate calcium, iron, and zinc ions (Jariwalla et al. 1990). Because of this activity, the consumption of phytate may be a health risk to those suffering from mineral deficiencies. However, in the case of healthy postmenopausal women, this characteristic may function as an antioxidant property, protecting against oxidative damage. Phytate's iron

chelating quality hinders iron's ability to participate in the Fention reaction and catalyze •OH formation (Hawkins et al. 1993), thus decreasing cellular injury as is seen in chronic disease and cancer. Phytate may decrease atherosclerotic lesion formation by preventing the absorption of iron, thus preventing lipid peroxidation (Ko and Godin 1991; Shamsuddin 1995) and oxidative damage to circulating proteins (Potter 1995). Supporting this theory, Porres et al. (1999) found that phytate in corn and soy was protective against lipid peroxidation in the colon of pigs with moderately high levels of dietary iron.

Phytate may also protect against CVD due to its ability to reduce blood lipids. Jariwalla et al. (1990) found that adding phytate to a cholesterol-enriched diet for six weeks significantly lowered serum TC and TG in rats; however, the mechanisms by which these occur are still unknown. Binding to zinc may offer a lipid-lowering mechanism by lowering the zinc-to-copper ratio, which is associated with high plasma cholesterol concentrations (Potter 1995; Jariwalla et al. 1990; Klevay 1977). In addition to these mechanisms, phytate may also effectively inhibit platelet aggregation, thus contributing to the prevention of thromboembolic events and CVD (Vucenik et al. 1999). Therefore, phytate shows promise as a therapeutic or preventive dietary approach to reducing chronic diseases.

The question still remains whether isoflavones, phytate, or other soy components, are needed to achieve optimal health benefits. The effect of isoflavones and phytate may simply be additive, synergistic, or complimentary to soy protein and/or other constituents (Sirtori 2001). To promote cardiovascular health, it may be more beneficial to consume soy products containing all the components rather than taking individual components. Consuming a diet low in saturated fat and cholesterol may also help to achieve optimal health

status (Sirtori 2001). The effects of aging are inevitable, but with current scientific progress, it may be possible to delay the aging process or at least make it more tolerable.

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SOY ISOFLAVONES AND PHYTIC ACID: EFFECTS ON BLOOD LIPIDS AND OXIDATIVE STRESS IN POSTMENOPAUSAL WOMEN

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ABSTRACT

Background: Postmenopausal women are at risk for developing cardiovascular disease (CVD) due to unfavorable blood lipid profiles and increased oxidative stress. Soy protein consumption may help protect against these risk factors.

Objective: To determine the effect of the soy protein components, isoflavones and phytate, on the risk of CVD in postmenopausal women.

Design: In a double-blind study, 55 postmenopausal women were randomly assigned to 1 of 4 soy protein isolate (SPI) treatments: low phytate, low isoflavone (LP/LI); normal phytate, low isoflavone (NP/LI); low phytate, normal isoflavone (LP/NI); or normal phytate, normal isoflavone (NP/NI). Subjects consumed 40 g soy protein/d for 6 wk. Blood lipids (total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], triacylglycerol [TG]) and oxidative stress indices (protein carbonyls [PC], oxidized low-density lipoproteins [oxLDL], 8-iso-prostglandin-$F_{2\alpha}$ [$PGF_{2\alpha}$]) were measured at baseline and 6 wk.

Results: Although the normal-isoflavone treatments showed a marginal effect on oxLDL, there was no overall significant effect on oxidative stress indicators. Both treatment groups

consuming normal isoflavone levels showed a significant decrease in TC ($p<0.05$) & LDL-C ($p<0.01$) after six weeks; however, the contrast between the low-isoflavone and normal-isoflavone groups was not significant (TC $p=0.16$, LDL-C $p=0.15$). The normal-phytate versus low-phytate treatments showed a trend toward decreasing PC and $PGF_{2\alpha}$; however, the differences were not significant.

Conclusions: Isoflavones, regardless of phytate content, may moderately reduce risk of CVD by favorably altering blood lipids. Phytate, regardless of isoflavone content, did not show a significant effect on either blood lipids or oxidative stress indicators.

KEY WORDS Postmenopausal women, Oxidative stress, Cardiovascular Disease, Soy Protein, Isoflavones, Phytate, Blood Lipids

INTRODUCTION

Men are usually at higher risk of developing cardiovascular disease (CVD) than women. After menopause, the risk for women becomes similar to that of men. The lack of estrogen may be responsible for affecting antioxidant systems, as well as lipid and lipoprotein concentrations (Goudev et al. 2000), both of which are implicated in the pathogenesis of CVD (Saito et al. 2003; Cooke et al. 2003).

Soy protein consumption has been shown to significantly decrease serum concentrations of TC, LDL-C, and TG (Vigna et al. 2000; Scheiber et al. 2001). This cholesterol-lowering activity is generally attributed to the isoflavones in soy. Adding soy protein with isoflavones to a low-fat diet for 3 months in hyperlipidemic men and postmenopausal women significantly ($p<0.01$) reduced TC and LDL-C (Jenkins et al. 2002).

Conversely, other studies with isoflavone-rich soy protein showed no effect on serum lipid concentrations (Dent et al. 2001). A 55-mg dose of isoflavones in men and postmenopausal women for 8 weeks showed no improvement in serum lipid concentrations (Hodgson et al. 1998). Additionally, Hsu et al. (2001) found that 150 mg of isoflavones per day for 6 months to normal postmenopausal women elicited no significant change in plasma lipids. These conflicting results may be due to subject selection, large interindividual variation, or various doses of isoflavones. Hence, the effect of isoflavones on serum lipids in humans remains unclear.

Soy isoflavones may also possess antioxidant properties, protecting against oxidation of LDL (Jenkins et al. 2000). Following a 12-week intervention with soy foods high in isoflavones, serum daidzein, genistein, and total isoflavone concentrations were positively correlated with LDL oxidation lag time in postmenopausal women (Scheiber et al. 2001). One theory is that isoflavones quench free radicals by donating a hydrogen atom to free radicals, thus making them less reactive (Mitchell et al. 1998). However, this has only been demonstrated in vitro. Another possible mechanism of isoflavones may be by increasing antioxidant concentrations. A 30-d administration of genistein (50 ppm) has been shown to increase antioxidant enzymes in mice (Cai and Wei 1996), and a 3-wk, 44-mg dose of genistein/d (40 g protein) raised the total antioxidant status in men (Rossi et al. 2000).

Phytate, another component of soy protein, was originally considered an anti-nutrient, but may be beneficial in some circumstances. Phytate has a stronger free radical quenching ability than isoflavones, and can bind minerals, such as iron, zinc, and calcium. Because of this phytate makes iron unavailable to participate in the Fention reaction and catalyze hydroxyl radical formation in vitro (Rao et al., 1991). Thus, phytate may prevent oxidative

damage, such as lipid peroxidation (Ko and Godin 1991; Porres et al. 1999) and atherosclerotic lesion formation (Potter 1995). Although phytate is known to be absorbed in rats (Sakamoto et al. 1993), a very small absorption was found in humans (Grases et al. 2001). Based on its antioxidant properties, even a small amount may protect against oxidative stress (Rao et al. 1991).

In addition, to its antioxidant activities, phytate has shown lipid-lowering activities. Jariwalla et al. (1990) found that adding phytate to a cholesterol-enriched diet for six weeks significantly lowered serum TC and TG in rats. However, data in humans are limited. Binding to zinc, and thus lowering the zinc-to-copper ratio, which is associated with high plasma cholesterol concentrations, may be responsible for the lipid-lowering effects (Potter 1995; Jariwalla et al. 1990; Klevay et al. 1977). The overall hypothesis of this study is that soy protein will reduce the risk of CVD, specifically that isoflavones will favorably alter blood lipid concentrations and that phytate will decrease oxidative stress indices.

SUBJECTS AND METHODS

Study Design

In this 6-week, double-blind study, 55 free-living postmenopausal women were randomly assigned to 1 of 4 soy protein isolate (SPI) treatments provided by the Solae Company (St. Louis, MO): low phytate and low isoflavone content (LP/LI, n=14); normal phytate and low isoflavones (NP/LI, n=13); low phytate and normal isoflavones (LP/NI, n=14); or normal phytate and normal isoflavones (NP/NI, n=14). All subjects were Caucasian, with the exception of one Asian woman. The women were supplied with two 20 g packets/d of protein (84 total packets) in a powder form. They were also given recipes to incorporate the powder into fruit smoothies or other foodstuffs. Two weeks prior to and

during the intervention, subjects were required to avoid all supplements, including vitamins, minerals, and herbal remedies. Subjects were also required to avoid phytate (Harland and Oberleas 1987; Reddy 2002) and isoflavone-rich (Stevens and Associates, Inc. 2001) foods during the intervention.

The phytate and aglycone contents, respectively, of each treatment per 40 g soy protein powder were as follows: LP/LI=0.22 g, 1.2 mg; NP/LI=0.64 g, 1.2 mg; LP/NI=0.22g, 85.8 mg; NP/NI=0.78 g, 84.6 mg. Alcohol extraction was used to prepare the low-isoflavone SPI, and phytase hydrolysis was used to prepare the low-phytate SPI.

Subject Selection

Postmenopausal women were recruited throughout central Iowa from April 2002 through November 2002. Approximately 300 women responded, and telephone interviews were conducted to screen the potential participants to ensure they met the inclusion and exclusion criteria. Subjects were included in the study if they were postmenopausal (last menses $\geq$12 months prior to intervention), healthy (no chronic disease or medication use), and had a body mass index (BMI) 19-34 kg/m^2. Women were excluded if they had a chronic disease, had a hysterectomy, had taken hormone therapy $\leq$12 months prior to intervention, and/or had used cigarettes or hormone creams $\leq$6 months prior to intervention. Based on these criteria, 57 women were qualified to participate. However, two of the women withdrew due to intolerable gastrointestinal side effects. The remaining 55 women completed the 6-week intervention with little or no gastrointestinal side effects.

Data Collection

Health and medical history, nutrition history, and soy food intake (Kirk et al 1999) were obtained at baseline using interviewer-administered questionnaires. Usual dietary

intake was also assessed at baseline with a food frequency questionnaire from Block Dietary Data Systems (Berkeley, CA). Overnight fasted blood samples were collected at baseline and week-6 and were frozen at -80°C until used. A standard reference laboratory (Quest Diagnostics, St. Louis, MO) analyzed serum and plasma for blood lipid profile (total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], triacylglycerol [TG]) and other blood chemistry analytes. Oxidative stress indices (protein carbonyls [PC], oxidized low-density lipoproteins [oxLDL], and 8-iso-prostglandin-$F_{2\alpha}$ [$PGF_{2\alpha}$]) were measured in the laboratory at Iowa State University. Protein carbonyls were measured using a slight modification of the procedure described in Reznick et al. (1992). Briefly, plasma was mixed with dinitrophenylhydrazine dissolved in hydrochloric acid (HCl), accompanied by blanks in HCl alone. Protein was then precipitated with 20% (w/v) trichloroacetic acid (TCA) and washed once with 10% TCA and three times with 5 ml of a 1:1 ethanol/ethyl acetate mixture. Finally, precipitates were dissolved in a 6 M guanidine-HCl solution. Absorbance was measured spectrophotomically at 380 nm. Plasma oxidized low-density lipoprotein was determined using an enzyme-linked immunoassay (ELIZA) kit from ALPCO Diagnostics (Windham, NH), and serum $PGF_{2\alpha}$ (free and esterified) concentration was determined using a direct ELIZA kit from Stressgen Biotechnologies (Victoria, BC Canada).

Statistical Analysis

Statistical analysis was performed with SAS (Version 8.0). Student t-test was used to determine the mean difference between baseline and week-6 for each treatment. The differences in percent change ([baseline concentrations - week-6 concentrations / baseline concentrations] * 100) among the treatments were analyzed using analysis of variance

(ANOVA) with Duncan's multiple range test. Contrast coding (LP vs NP and LI vs NI) was used to determine the individual effect of phytate and isoflavones. Pearson correlation analysis was used to determine the relationships among the risk factors. Statistical significance was set at $p<0.05$.

RESULTS

Compliance

Compliance was based on the number of packets returned at the week-6 visit. Most of the women consumed 100% of their packets. However, four subjects returned, 2, 3, 4, and 8 packets and two subjects requested 3 and 4 extra packets. These subjects were evenly distributed across the treatment groups.

Subject Characteristics

The age of subjects ranged from 47-72 years. The range of time since menopause was 1-20 years with a mean of 6.4 years. Thirteen subjects had smoked in earlier years, six subjects had experienced cancer (skin [n=4], breast [n=1], cervical [n=1]) but were all in remission, and one woman had two previous strokes that were not related to CVD. There were no other reported cardiovascular events or diagnosis of CVD.

Twenty-eight and twenty-six of the subjects conveyed their health as good and excellent, respectively, whereas one reported her health as fair. Education levels were high school (sixteen), college (twenty-nine), and post-college (ten). Thirteen of the participants stated they had experienced iron deficiency at least once in their lifetime due to malnutrition, pregnancy, menstruation, or the onset of menopause. Thirty-six subjects (eight to ten per treatment) reported regular use of a multivitamin, and two subjects (LP/LI=1, LP/NI-1) reported regular use of an iron supplement. Thirty-five subjects (seven to eleven per

treatment) reported they were consuming soy or soy products prior to the intervention, although most conveyed it was irregular.

Subjects' descriptive and daily nutrient intakes are reported in Table 1. None of the descriptive characteristics at baseline were significantly different among the treatment groups. The dietary intakes of macronutrients were within normal ranges and did not significantly differ among the treatment groups. Overall, intake of selected antioxidants (vitamins A, C, and E) was within the recommended allowance in each treatment group. Although vitamin C values were higher in two treatments (NP/LI, LP/NI), the difference was not significant.

Oxidative Stress Indices

Oxidative stress indices before and after intervention are presented in Table 2. The protein carbonyl concentrations were very low (0.2 nmol/mg protein), and there was no significant change from baseline in any treatment. At baseline, the $PGF_{2\alpha}$ concentration in the NP/LI treatment group was significantly different ($p=0.05$) from that in the LP/LI treatment group, but not from the other two groups. For each of the treatments, the $PGF_{2\alpha}$ concentration decreased (Figure 1), but this was not significant. Although, the change in $PGF_{2\alpha}$ concentration in the NP/LI treatment group was 14-29% higher than in the other three groups, it was not significant. The degree of reduction in $PGF_{2\alpha}$ seemed to be related to the initial concentration, since the maximum decline was seen in the group that had the highest mean value at baseline. The mean oxLDL concentration ranged from 66-81 U/L in each treatment. Similar to $PGF_{2\alpha}$, there was a reduction in each treatments group. The 10 U/L modest reduction in the LP/NI group ($p=0.052$) was greater than the 4-6 U/L reduction in the other groups (Figure 1). However, the differences were not significant based on ANOVA.

Overall, oxLDL concentrations decreased to a greater extent in the treatments containing normal isoflavone levels (7-10%) compared to the treatments containing low isoflavone levels (3-7%). Although PC decreased in each treatment except LP/NI, neither the reductions from baseline nor the differences among the treatments was significant. In general, the normal-phytate groups reduced PC 6-7% (Figure 1).

To determine the effect of isoflavones and phytate individually, contrast coding was used to test which contrast (LP vs. NP; LI vs. NI) had a significant effect on each risk factor. The normal-phytate containing treatments reduced PC 14 pmol/mg compared to 3 pmol/mg in the low-phytate containing treatments; however, this contrast was not significant ($p=0.18$) (Figure 3). Similarly, $PGF_{2\alpha}$ decreased 265 pg/mL in the normal-phytate treatments compared to the 163 pg/mL decrease in the low-phytate treatments, but this contrast was not significant ($p=0.68$). In contrast, oxLDL was reduced to a greater extent in the normal-isoflavone containing treatments (7.9 U/L) than in the low-isoflavone containing treatments (4.3 U/L), although this difference was also not significant ($p=0.4$).

Blood Lipid Concentrations

Blood lipid values at baseline and week-6 are presented in Table 2. Baseline TC ranged from 222-235 mg/dL among all treatments. Total cholesterol concentrations declined after six weeks in all treatments; however, the normal-isoflavone groups had a greater reduction (14-16 mg/dL) than the low-isoflavone groups (Figure 2). In the LP/NI and NP/NI treatment groups, respectively, TC significantly declined ($p=0.04$ and $p=0.01$) from baseline. Similarly, LDL-C decreased with treatment in all groups (Figure 2), but the only significant reductions were noted in LP/NI ($p=0.006$) and NP/NI ($p=0.002$). The initial TG concentration in NP/LI was significantly higher than the other treatment groups. However,

there was no significant change from baseline in any treatment group. Similar to TG, there were no significant changes is HDL-C concentration in any treatment group, although there was a significant difference at baseline between NP/LI and NP/NI.

Contrast coding revealed that the normal-isoflavone groups had a decline in TC by 14.8 mg/dL, whereas the decline in TC in the low-isoflavone groups was 5.9 mg/dL (Figure 4). Similarly, LDL-C decreased to a greater extent in the normal-isoflavone groups (15.0 mg/dL) than in the low-isoflavone groups (6.4 mg/dL). However, neither of these differences was significant. Contrast coding illustrated that phytate had no effect on blood lipid concentrations.

Correlation of CVD Risk Factors

Correlations among baseline BMI, oxidative stress indices, and blood lipid measures are shown in Table 4. The highest positive correlations ($p<0.0001$) were observed between TG and $PGF_{2\alpha}$, as well as among TC, oxLDL, and LDL-C. In addition, BMI highly correlated with TG ($p<0.001$), $PGF_{2\alpha}$ ($p<0.05$), and oxLDL ($p<0.05$). High-density lipoprotein cholesterol negatively correlated with TG ($p<0.0001$) and BMI ($p<0.0001$), as well as with $PGF_{2\alpha}$ ($p<0.001$) and oxLDL ($p<0.05$).

DISCUSSION

The soy health claim from FDA recommends a daily intake of 25 g soy protein (Stein 2000), based on the amount needed to favorably alter blood lipid profiles. In this study, we chose to use 40 g/d soy protein in order to determine whether a higher intake would elicit beneficial effects on blood lipid profiles, as well as on oxidative stress indices in postmenopausal women, who are at high risk for CVD. This amount was also chosen based on previous human studies that investigated health benefits of soy protein (Dent et al. 2001;

Teede et al. 2001; Potter et al. 1998; Swain et al. 2002). In addition, to obtain the amount needed to show beneficial effects of dietary phytate, the subjects needed to consume such an amount of soy protein. Although, the amount of phytate provided daily from soy protein (0.64-0.78 g) was still lower than the amount used in previous rat studies (9% w/w) that showed beneficial effects (Jariwalla et al. 1990).

Subjects were recruited throughout the summer and fall seasons, which potentially might have affected exercise habits and/or dietary intake patterns during the intervention. However, there was no change in BMI with intervention in each treatment, suggesting that the seasonal variation in either dietary intake or physical activity had no effect. In addition, all the subjects consumed the same amount of protein, thus we did not expect any difference in the dietary intakes among the treatments.

The NP/LI group had higher oxidative stress in terms of $PGF_{2\alpha}$, oxLDL, (Table 2) HDL-C, and TG (Table 3) concentrations. Although, the change in $PGF_{2\alpha}$ was more evident in the NP/LI group, the percent change was lower than that in the LP/LI and NP/NI groups (Figure 1). In contrast to other published studies, the reduction in oxLDL with isoflavones was modest in our study. Exner et al. (2001) found that genistein effectively prevented glucose-mediated LDL oxidation in vivo. In addition, serum daidzein, genistein, and total isoflavone concentrations positively correlated with LDL oxidation lag time in postmenopausal women following a 12-week intervention with soy foods high in isoflavones (Scheiber et al. 2001). It may be possible that the sample size in our study may have been too small to detect a significant change in oxLDL. The effect of isoflavones on reducing oxLDL may also depend on the oxidative status of the subjects at baseline. Although 33 g/d soy protein for one month significantly decreased oxLDL in hyperlipidemic subjects (Jenkins

et al. 2000), it is not clear whether this effect can be found in normolipidemic subjects. In our study, there was a correlation between blood lipids and oxLDL, but the blood lipid concentrations were not high enough in our subjects to clarify them as hyperlipidemic.

Similar to the effects on oxLDL, but to a smaller extent, all of the treatment groups experienced a decline in $PGF_{2\alpha}$ concentrations, suggesting a soy protein effect. Phytate contrast notably decreased $PGF_{2\alpha}$ by almost two-fold; however, neither of the contrasts had a significant effect (Figure 3). Although it was modest, the normal-phytate groups had a greater effect on PC than the normal-isoflavone groups. However, this may have been due to the overall increase in the LP/NI treatment group (Figure 1), thus comparing the low-phytate and normal-phytate treatments caused a larger difference in the means. Phytate has a strong chelating ability for iron under in vitro conditions (Rao et al. 1991); however, it is possible that in vivo it does not reduce the free radical formation. It is also possible that the amount of phytate in the soy protein was not enough to see an effect, since absorption is low in humans (Grases et al. 2001).

The modest effect of the normal- versus low-isoflavone treatments on reducing TC and LDL-C may have been due, in part, to the mean initial concentrations in TC (227-235 mg/dL) and LDL-C (144-151 mg/dL), although these values may not be considered to be that high. It has been demonstrated that soy isoflavones exert more pronounced affects in hypercholesterolemic than normolipidemic subjects (Vigna et al. 2000). Additionally, consuming isoflavone soy-food diets for 1 month reduced blood lipids, homocysteine concentrations, and blood pressure in hyperlipidemic men and postmenopausal women (Jenkins et al. 2002), whereas Hsu et al. (2001) found that supplementing 150 mg of isoflavones per day for 6 months to normal postmenopausal women educed no significant

change in plasma lipids. Dent et al. (2001) also found that isoflavone-rich soy protein for 24 wk had no effect on circulating lipids in normal perimenopausal women.

Although the differences were not significant, the increases in HDL-C for LP/LI and LP/NI were 2.1 and 7%, respectively (Figure 2). However, the low-isoflavone treatments, combined, produced an increase that was modestly different from the reduction caused by the normal isoflavone groups (Figure 4). In contrast, Goodman-Gruen et al. (2001) found that isoflavone intake in postmenopausal women was positively associated with HDL-C concentrations. The mean baseline TG concentration in the NP/LI treatment group was significantly and inexplicable higher (168 mg/dL) than in the other groups (100-112 mg/dL) (Table 3). However, the change from baseline was not significantly different among the treatments. Tonstad et al. (2002) saw a similar increase in TG concentrations following a 24-wk, 30-g and 50-g soy protein/d diet in men and women. In contrast to the modest effect of isoflavones, phytate had no effect on blood lipid concentrations. These results again do not support the rat studies that have shown lipid lowering effects of phytate. However, Jariwalla et al. (1990) used 9% (w/w) phytate, which was a higher amount than was given to our subjects, and they added the phytate to a cholesterol-enriched diet, which may have affected the results. Also, rats absorb phytate more readily than humans (Sakamoto et al. 1993; Grases et al. 2001).

The positive correlation of BMI with oxidative stress markers, oxLDL and $PGF_{2\alpha}$ (Table 4) is noteworthy. Vasankari et al. (2001) found a similar correlation between BMI and oxLDL, whereas Suzuki et al. (2003) found no correlation between the two markers. Baseline BMI was also negatively correlated with HDL-C (Table 4), similar to what has been reported in other studies (Bautista-Castano et al. 2003). Additionally, a negative correlation

between HDL-C and fat mass has also been reported (Ito et al. 2003), suggesting unfavorable HDL-C concentrations in overweight or obese individuals. Lowering BMI may be beneficial to reduce oxidative stress and HDL-C.

In conclusion, our study showed that the treatments containing normal isoflavone levels only had a modest change on oxidative stress markers and blood lipids. Also, isoflavones may have a greater effect on favorable altering circulating lipids and lipoproteins than phytate. In contrast, most of the oxidative stress indices were reduced more evidently with the phytate treatments. Compared to studies that only looked at total antioxidant status (Swain et al. 2002; Rossi et al. 2000), this study included many oxidative stress indicators to assess damage to proteins and lipids, which may be more indicative of oxidative damage. Based on the modest effects noted in this study, future studies with a larger number of subjects may be needed to produce more significant effects from isoflavones and phytate.

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TABLE 1

Baseline characteristics of subjects (mean [min-max])

	Treatment			
	LP/LI (n=14)	**NP/LI (n=13)**	**LP/NI (n=14)**	**NP/NI (n=14)**
Age (y)	56 [49-70]	59 [53-69]	58 [47-72]	60 [50-70]
Weight (kg)	71 [58.2-93.6]	72.7 [59.0-96.8]	72.7 [55.2-92.5]	69.2 [52.3-92.4]
Height (m)	1.7 [1.5-1.8]	1.6 [1.5-1.7]	1.7 [1.6-1.7]	1.7 [1.6-1.7]
BMI (kg/m^2)	25.9 [18.6-32.9]	27.9 [21.2-33.9]	26.5 [19.9-32.0]	25.3 [21.3-33.6]
Dietary Intake/d*				
Energy (kcal)	1702 [515-2559]	1766 [763-2708]	1769 [877-2827]	1647 [1144-2595]
Protein (g)	65 [22-104]	68 [30-108]	71 [31-122]	63 [39-103]
Carbohydrate (g)	217 [53-412]	239 119-405]	213 [85-339]	204 [146-328]
Fat (g)	66 [25-96]	64 [22-113]	75 [36-139]	67 [30-105]
Saturated Fat (g)	21 [7-37]	18 [6-30]	21 [12-38]	18 [9-26]
Vitamin C (mg)	103 [42-192]	155 [99-312]	130 [43-211]	116 [50-170]
Vitamin E (α-TE)	9 [3-15]	11 [5-19]	11 [6-19]	10 [5-21]
Vitamin A (RE)	1753 [305-3934]	1734 [565-3065]	1570 [698-5107]	1161 [656-2238]

LP/LI = low phytate, low isoflavone; NP/LI = normal phytate, low isoflavone;
LP/NI = low phytate, normal isoflavone; NP/NI = normal phytate, normal isoflavone

*Selected nutrients assessed using Food Frequency Questionnaire (Block Dietary Data Systems)

There were no significant differences for any of these characteristics at baseline among the treatment groups

TABLE 2

Oxidative stress measures at baseline and 6 weeks (Mean ± SD)

Treatment	Time	PC (pmol/mg)	$PGF_{2\alpha}$ (pg/ml)	oxLDL (U/L)
LP/LI	**Base**	204 ± 54	4118 ± 573[a]	66.4 ± 15.9
	Post	196 ± 49	3978 ± 654	62.2 ± 17.9
	Difference[+]	-9 ± 31	-139 ± 653	-4.2 ± 9.2
NP/LI	**Base**	197 ± 55	5327 ± 1782[b]	81.0 ± 25.3
	Post	181 ± 40	5000 ± 1200	76.6 ± 24.4
	Difference[+]	-16 ± 29	-327 ± 1397	-4.3 ± 17.6
LP/NI	**Base**	183 ± 39	4471 ± 1183[ab]	77.9 ± 29.4
	Post	185 ± 42	4286 ± 1040	67.9 ± 18.8
	Difference[+]	2 ± 31	-186 ± 893	-10.0 ± 17.5
NP/NI	**Base**	190 ± 35	4678 ± 1366[ab]	75.1 ± 21.4
	Post	178 ± 43	4411 ± 992	69.3 ± 24.0
	Difference[+]	-12 ± 24	-207 ± 637	-5.9 ± 18.5

LP/LI = low phytate, low isoflavone; NP/LI = normal phytate, low isoflavone; LP/NI = low phytate, normal isoflavone; NP/NI = normal phytate, normal isoflavone

PC = protein carbonyls; oxLDL = oxidized low-density lipoproteins; $PGF_{2\alpha}$ = 8-iso-prostglandin-$F_{2\alpha}$

[+]Mean difference (Week 6 - Baseline)

[a,b,ab] Superscripts refer to differences among the treatments for the same time point

Baseline values among the treatments were not different except for $PGF_{2\alpha}$

Differences from baseline to week-6 for each treatment were not significant

TABLE 3

Blood lipid measures at baseline and 6 weeks (Mean ± SD)

		TC (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)
LP/LI	**Base**	223 ±31	138 ±27	63 ±17[ab]	112 ±69[a]
	Post	215 ±33	129 ±29	65 ±14	101 ±55
	Difference[+]	-8 ±18	-9 ±19	3 ±7	-11 ±47
NP/LI	**Base**	225 ±31	139 ±32	53 ±10[b]	168 ±95[b]
	Post	221 ±36	135 ±28	53 ±10	167 ±77
	Difference[+]	-4 ±31	-4 ±33	1 ±6	-1 ±56
LP/NI	**Base**	227 ±35	144 ±30	62 ±13[ab]	104 ±43[a]
	Post	213 ±30	131 ±27	59 ±14	117 ±63
	Difference[+]	-14 ±23*	-14 ±16**	-3 ±9	12 ±32
NP/NI	**Base**	235 ±40	151 ±38	65 ±11[a]	100 ±51[a]
	Post	220 ±38	134 ±36	64 ±14	106 ±77
	Difference[+]	-16 ±20*	-16 ±16**	-1 ±7	7 ±39

LP/LI = low phytate, low isoflavone; NP/LI = normal phytate, low isoflavone; LP/NI = low phytate, normal isoflavone; NP/NI = normal phytate, normal isoflavone

TC = total cholesterol; LDL-C = low-density lipoprotein; HDL-C = high-density lipoprotein; TG = triacylglycerol

[+]Mean difference (Week 6 - Baseline)

[a,b,ab] Superscripts refer to the differences among the treatments for the same time point

* $p<0.05$, ** $p<0.01$, *** $p<0.005$

Baseline values among the treatments were not different except for HDL-C and TG

There was a significant decrease from baseline to week-6 in the LP/NI and NP/NI groups for TC and LDL-C

TABLE 4

Intercorrelations of baseline values [correlation coefficient (p-value)]

	$PGF_{2\alpha}$	oxLDL	TC	LDL-C	HDL-C	TG	BMI
PC	0.15	0.12	0.03	-0.05	0.00	0.18	0.10
$PGF_{2\alpha}$		0.25	0.17	0.04	-0.44[c]	0.74[d]	0.33[a]
oxLDL			0.61[d]	0.62[d]	-0.32[a]	0.39[b]	0.34[a]
TC				0.94[d]	0.07	0.20	0.14
LDL-C					-0.06	0.05	0.17
HDL-C						-0.65[d]	-0.52[d]
TG							0.45[c]

LP/LI = low phytate, low isoflavone; NP/LI = normal phytate, low isoflavone; LP/NI = low phytate, normal isoflavone; NP/NI = normal phytate, normal isoflavone

TC = total cholesterol; LDL-C = low-density lipoprotein; HDL-C = high-density lipoprotein; TG = triacylglycerol

PC = protein carbonyls; oxLDL = oxidized low-density lipoproteins; $PGF_{2\alpha}$ = 8-iso-prostglandin-$F_{2\alpha}$

[a] $p<0.05$

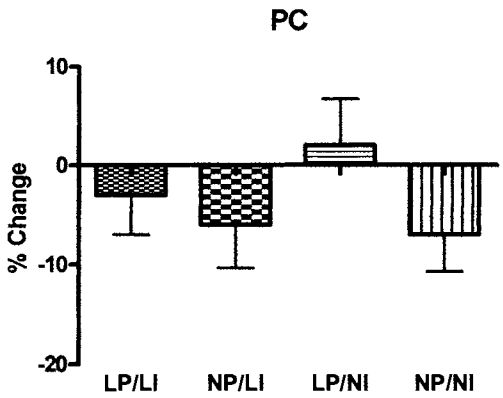
[b] $p<0.01$

[c] $p<0.001$

[d] $p<0.0001$

Figure 1: Percent Change in Oxidative Stress Indices

A



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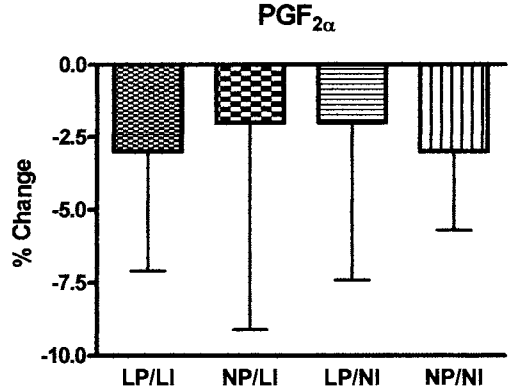

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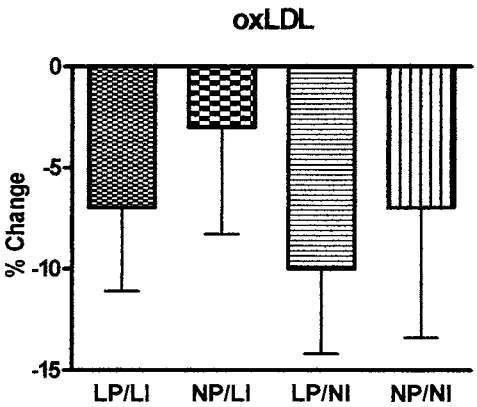
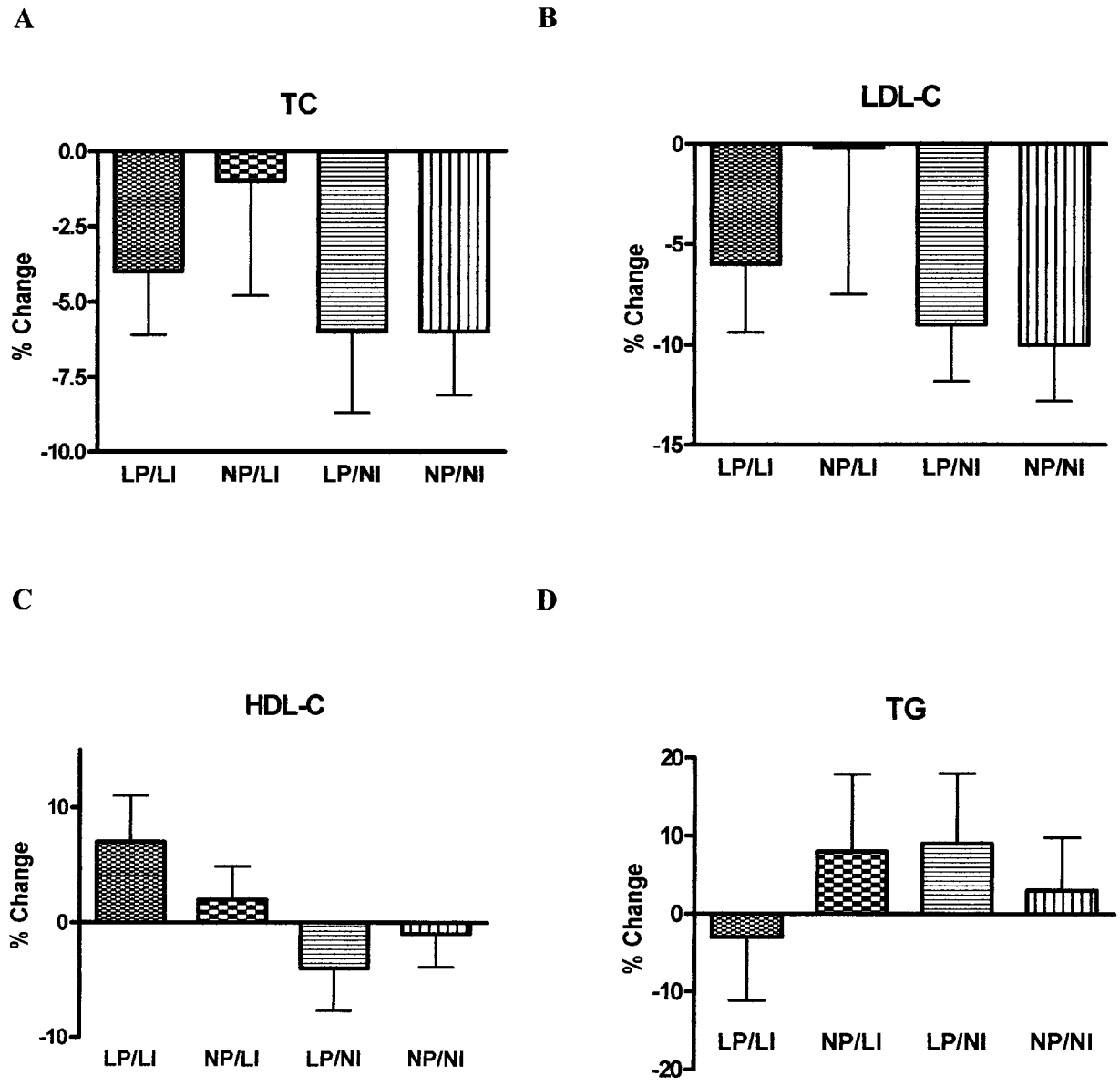


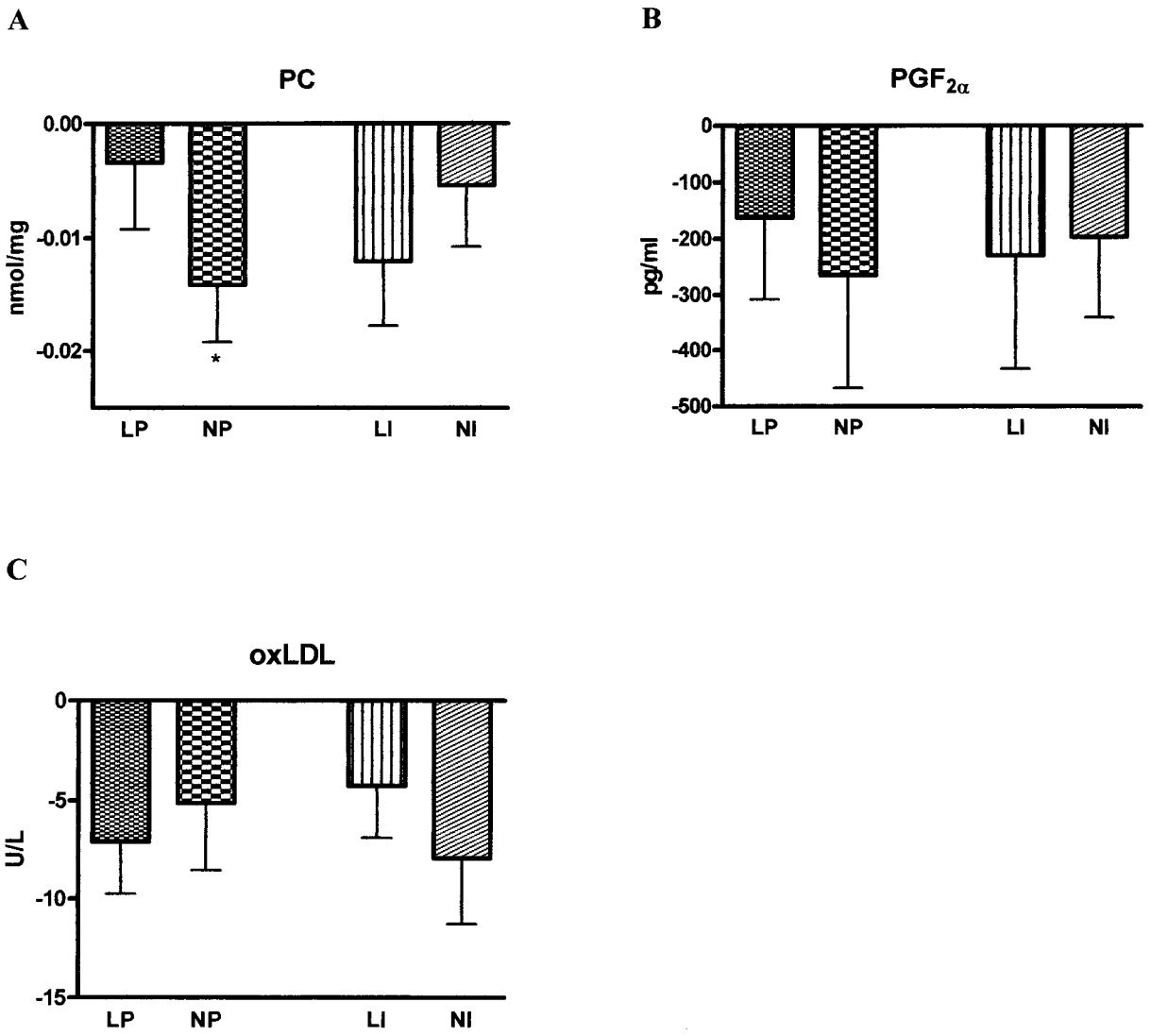
Panel A = protein carbonyls (PC), Panel B = 8-iso-prostglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$), Panel C = oxidized low-density lipoproteins (oxLDL)
%Change = [(Post-Base)/Base]*100
Percent change among the treatments are not significantly different for any of the indices.

Figure 2: Percent Change in Blood Lipids



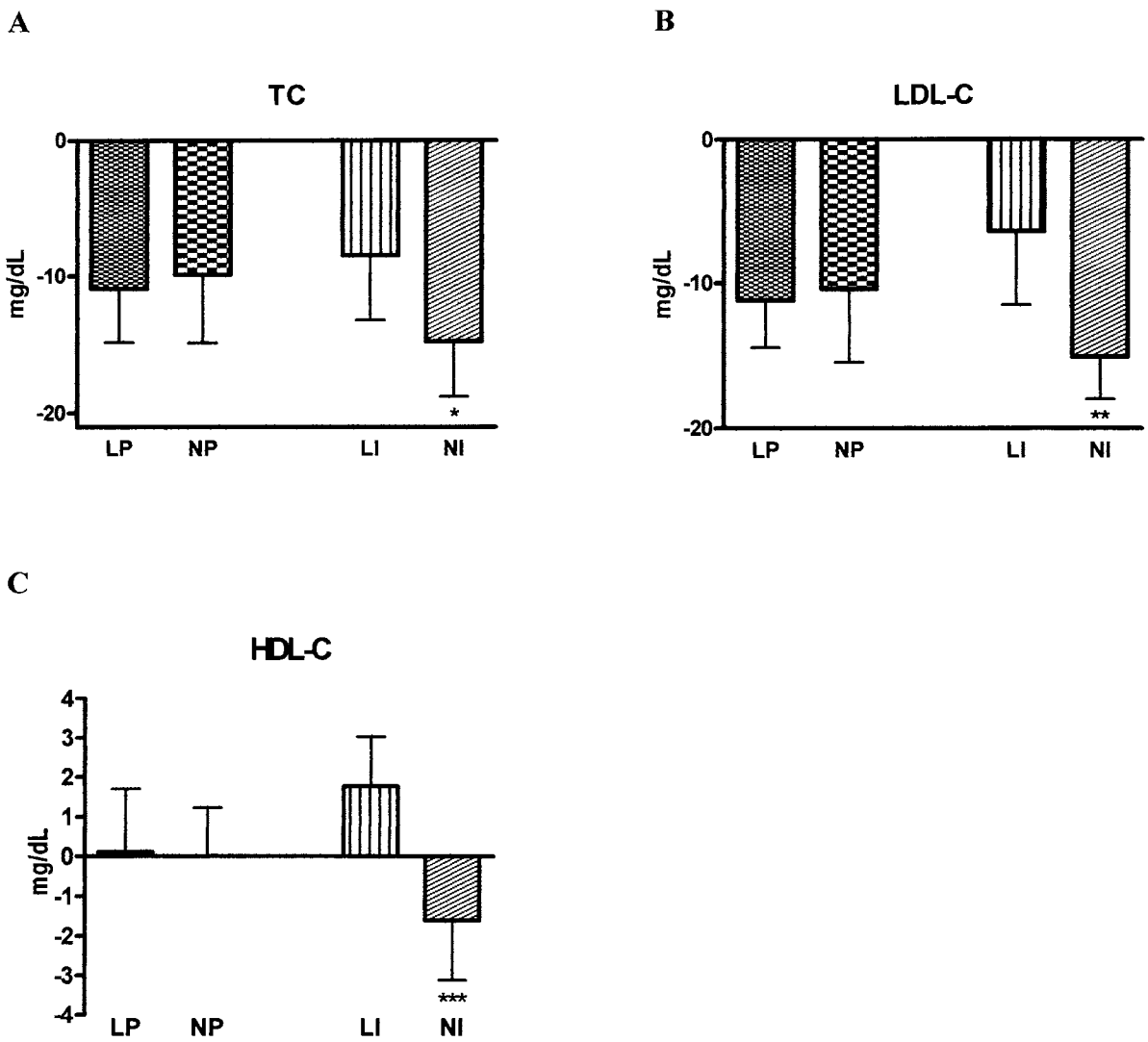
Panel A = total cholesterol (TC), Panel B = low-density lipoprotein cholesterol (LDL-C), Panel C = high-density lipoprotein cholesterol (HDL-C), Panel D = triacylglycerol (TG)
%Change = [(Post-Base)/Base]*100
Percent change among the treatments are not significantly different for any of the indices.

Figure 3: Contrast Coding for Oxidative Stress Indices



Panel A = protein carbonyls (PC), Panel B = 8-iso-prostglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$),
Panel C = oxidized low-density lipoproteins (oxLDL)
LP = LP/LI and LP/NI, NP = NP/LI and NP/NI, LI = LP/LI and NP/LI,
NI = LP/NI and NP/NI
***p=0.1751**
There was no significant difference for either of the contrasts (LP vs NP or LI vs NI) for all of the indices, based on contrast coding.

Figure 4: Contrast Coding for Blood Lipids Measures



Panel A = total cholesterol (TC), Panel B = low-density lipoprotein cholesterol (LDL-C), Panel C = high-density lipoprotein cholesterol (HDL-C)
LP = LP/LI and LP/NI, NP = NP/LI and NP/NI, LI = LP/LI and NP/LI, NI = LP/NI and NP/NI
***p=0.1623, **p=0.1477, ***p=0.0991**
There was no significant difference for either of the contrasts (LP vs NP or LI vs NI) for all of the indices, based on contrast coding.

GENERAL CONCLUSIONS

In summary, the results of this study suggest that soy protein containing normal isoflavone amounts have a moderate effect on reducing blood lipid concentrations and oxidative stress indices. However, the changes elicited by the isoflavones were not significant, suggest there may be other soy components responsible for the lipid-lowering and antioxidant effects of soy. It may also be that isoflavones have an additive effect with soy protein and/or other constituents rather than an individually effect. Phytate elicited similar results on oxidative stress indices, causing only marginal reductions with no significant difference. With regard to blood lipids, though, phytate had no effect.

The sample size in this study may have been too small to obtain significant effects, thus recruiting a larger number of subjects may be beneficial in future research. Also, future studies should increase the time of intervention in order to test sustainability of the results.